**Letter to the Editor**

**Bypassing the "Species Barrier" with Carbohydrate-altered Interferon from Leukocytes**

William A. Carter

Department of Medical Viral Oncology, Roswell Park Memorial Institute and State University of New York at Buffalo, Buffalo, New York 14263

**ABSTRACT**

Interferon, a glycoprotein with demonstrated antitumor and antiviral properties, can be obtained from cells of different species, and some of these interferons can be effective across species lines. I hypothesize that the ability to cross or not to cross species lines lies in the carbohydrate moiety, and cross-species biological activity is a property of the polypeptide. This would represent one of the first major roles uncovered for glycosylation in any biological system. If this hypothesis is correct, then it should be possible to use animal-derived interferon as a large-scale, inexpensive source to treat human diseases.

**INTRODUCTION**

Interferon, produced by different cell types, is a glycoprotein that has antiproliferative activity. It is being tested as a therapeutic agent in a variety of human diseases including malignant tumors (6, 23, 32). Clinical studies are limited by the expense and amount of interferon available which also prevents therapeutic trials in more types of neoplastic disease. Long-term, intensive interferon therapy may be valuable since its antitumor effect is based on different mechanisms than conventional chemotherapeutic agents (35).

Current procedures for production of interferon for use in humans involve human cells because of an apparent species specificity of interferon as originally described in 1957 (16). This severely limits the supplies of interferon for clinical trials, and only a few laboratories can currently produce human interferon in any significant amounts.

Animal-derived proteins are more readily available, inexpensive, and widely used already in clinical practice. Bioactive preparations from animal sources include insulin, thyrotrophic hormone, adrenocorticotropic hormone, and others. These polypeptides demonstrate a preservation of amino acid sequences crossing species lines with maintenance of activity. For example, comparative studies of insulin including that derived from a primitive vertebrate, the Atlantic hagfish, indicate preservation of peptide structure in the bioactive region throughout the entire history of vertebrate evolution (9). Interferon may have a similar preservation of a bioactive peptide.

Although the concept that interferons are glycoproteins with a restricted, species-specific activity is widely accepted, various exceptions have been reported, e.g., the pronounced effect of human leukocyte interferon on heterologous cells observed by Desmyter and Stewart (10) and Gresser et al. (11). Recently, porcine and bovine carbohydrate-altered interferons obtained from leukocytes were found to have increased efficacy in protecting human cells from viral challenge (5).

This suggests a relationship between the function of interferon and its glycosylation; namely, the structural homology and the cross-species activity may be masked by a part or all of the oligosaccharide. It implies that carbohydrate-altered animal interferons should be active in man whenever there is homology of a bioactive polypeptide. Chany (7) has advanced a model that the receptor system for homologous interferons may recognize its carbohydrate moieties; however, Bridgen et al. (4) and Stewart et al. (31) have observed an apparent dispensability of the carbohydrates for biological activity of interferon based on both their chemical and enzymatic removal from the polypeptide.

The antiviral activity of porcine, bovine, and equine leukocyte interferon is several-fold higher when measured on human cells than on their homologous cells (5). By contrast, interferon derived from animal fibroblasts does not display cross-species bioactivity; these interferons bind to lectin columns such as concanavalin A (8). Human fibroblast interferon is extensively glycosylated, and o-mannose, L-fucose, and sialic acid residues have been identified (17). It is at least 1000-fold less active on most heterologous cells than is human leukocyte interferon. The lack of retention of leukocyte interferon preparations (human and nonhuman) on various lectin columns is evidence of different oligosaccharides.

A specificity for binding of other serum glycoproteins to membrane receptors has been previously reported (15). Also, across species lines, membrane receptors for glycoproteins are known to differ in terms of which terminal sugar residue is recognized and bound (19). Even though sialic acid is present on human fibroblast interferon and missing on leukocyte interferon, the presence of peripheral sugars on fibroblast interferon does not account for the species specificity since when synthesized in the presence of 2-deoxyglucose, it is still species specific.2 Deoxyglucose blocks synthesis of peripheral sugars (29) and may leave part of the oligosaccharide core intact. Therefore, the core sugars (e.g., mannose and N-acetylglucosamine) of fibroblast interferons are probably more important residues in masking the cross-species activity. All interferon molecules (human or nonhuman) which bind to concanavalin A display strongly hydrophobic properties. This degree of hydrophobicity is consistent with an increased number of aromatic amino acids on the molecular surface (33). Glycosylation usually increases molecular surface hydrophilic properties.

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2 W. A. Carter, Unpublished data.
properties although this depends to a great extent on the nature of the sugar residues; the presence of ionically charged sialic acid is more hydrophilic than that of mannose. Binding to concanavalin A may indicate increased glycosylation of fibroblast interferons with the nature of sugar residues in part responsible for the decreased hydrophilic nature. Glycosylation probably also alters the tertiary structure of fibroblast interferons so as to displace hydrophobic amino acid residues from the interior to the exterior of the molecule. In effect, having a decrease of hydrophilic sugars promotes a change in tertiary structure so that more hydrophobic amino acids are closer to the molecular surface.

Tunicamycin (34) has been used to demonstrate that glycosylation alters the physical shape of immunoglobulins (14). Human immune interferon produced by exposure of lymphocytes to various mitogens exists in several molecular forms (26) which are apparently due to variations in glycosylation. These forms bind hydrophobically to various immobilized polynucleotides, e.g., polyuridylic acid. However, when immune interferon is produced in the presence of tunicamycin, only one form is synthesized (24). This form appears similar to the leukocyte interferon; namely, it has fewer surface hydrophobic groups, less polynucleotide binding, and no binding to lectins. This carbohydrate-depleted form may be expected to display protection of nonhuman cells similar to the leukocyte-derived interferon (10, 11). Thus, although glycosylation is of no apparent consequence for secretion of interferon or some other glycoproteins (13), its impairment does change various physicochemical properties. The increased hydrophobicity which occurs after glycosylation of interferon could be due in part to surface exposure of hydrophobic polypeptides located within the bioactive site required for both recognition by the cell receptor and action. The bioactive site becomes distorted. Hydrophobic peptide residues are often part of the bioactive site of hormones (27) and provide a major driving force for interaction of the protein with its cell surface receptor. Distortion of these residues would alter the binding properties of interferon and its induction of synthesis of mediator proteins, all of which probably depend on tertiary structure. An alteration in interaction with surface receptor also explains why the species specificity is not observed at the subcellular level. In cell-free systems, the interferon-induced inhibitors are functionally interchangeable (1).

An evolutionary constraint may exist on the structure of cell membrane receptor proteins of many polypeptide hormones (3) and could account for the "best fit" of a protein for its receptor not always occurring even in the same species. Examples of enhanced bioactivity of proteins in heterologous cells already exist in clinical medicine; for example, salmon calcitonin has high activity in man when compared with human calcitonin. While glycosylation seems to alter the "fit" in the interaction between interferon and receptor on heterologous cells, it may enhance activity in homologous cells: purified fibroblast interferon has very high specific activity (2) near values theoretically attainable as discussed by Joklik (18). When synthesized in the presence of inhibitors of glycosylation, Havell et al. (12) demonstrated that the molecular weight of human fibroblast interferon decreases by about 5000 indicating that some 25% of its molecular weight is due to oligosaccharide units. Even small modifications of the bioactive site (adding various groups or inducing configurational changes) can alter protein affinity for receptor (9, 27). It will be valuable to determine whether fully glycosylated fibroblast interferons fail to bind to receptor on heterologous cells or occupy receptor but fail to activate.

During synthesis of the leukocyte interferon glycopeptide, certain alterations in its glycosylation must occur which preserve cross-species activity of the polypeptide. Biosynthesis of glycoproteins involves the en bloc transfer of a large oligosaccharide chain containing N-acetylgalactosamine, mannose, and glucose to the asparagine residue of a polypeptide (28). The chain is then processed by removal of several residues followed in the Golgi by sequential enzymatic addition of the peripheral sugars (N-acetylgalactosamine, galactose, and sialic acid) to form the final oligosaccharide. With leukocyte interferon, an enzymatic defect in processing probably occurs which results in a glycoprotein with an altered oligosaccharide core. Enzymatic defects in the processing step have been detected in clones of Chinese hamster ovary cells (22), and these cells are greatly altered in their lectin-binding properties. Part of the carbohydrate chain is probably added during the synthesis of the polypeptide (21, 30), and this may magnify its effect on the shape of the molecule since the glycoprotein (e.g., interferon) has not yet assumed a more rigid structure as when synthesis is complete.

Stepwise stripping of sugar residues from fibroblast interferons with specific glycosidases (20, 21) should alter configuration and modulate binding with receptors on heterologous cells; activation of these cells might then occur. Specifically, the use of either mannosidases or endoglycosidases which attack the core region of the interferon oligosaccharide may resurrect activity.

Efficacious forms of animal interferon could be produced from leukocytes obtained at the slaughter house. One obvious major advantage is the availability of sufficient leukocytes to provide for many long-term clinical studies of the antiproliferative activity of interferon. Estimates based on the present annual slaughter of pigs in the United States alone indicate that even this one source would greatly exceed the currently available supplies of interferon from all human cell sources. This would permit more extensive pharmacokinetic studies of interferon using in vivo primate models. Clinical use of animal leukocyte interferons could be safe especially due to the lack of occult human viruses (36). Viral infections in humans trigger the production of different antigenic forms of interferon (25, 26). These human interferon forms are not recognized by the immune system as foreign; i.e., there exists cross tolerance within the human species for all the human interferon forms. Small differences in the primary structure which differentiate interferon from one species to another may also escape the immune system, thereby extending human immunological tolerance to carbohydrate-depleted interferons from nonhuman sources. However, the question of antigenicity obviously remains to be resolved. Since the use of nonhuman leukocyte interferon may have significant scientific and clinical value, further investigation of these large-scale sources is most timely.

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